Sequence variation in the hpd gene of noncapsulated Haemophilus influenzae isolated from patients with chronic bronchitis

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Sequence variation in the hpd gene of nonencapsulated *Haemophilus influenzae* isolated from patients with chronic bronchitis

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Abstract

The molecular diversity of protein D of nonencapsulated *Haemophilus influenzae* strains isolated from persistently infected patients with chronic bronchitis was studied by sequencing the hpd gene of four independently obtained isolates. The nucleotide (nt) sequences of the hpd genes of two strains were identical. The other two hpd sequences showed nt substitutions which were mostly synonymous. As a consequence the deduced amino acid (aa) sequences differed from the consensus sequence only by a few aa. No changes in the hpd genes were observed among the four variants of the four strains persisting in chronic bronchitis patients for 9, 11, 8 and 3 months, respectively, although variation in their major outer membrane proteins P2 and P5 occurred. We conclude that the hpd gene is conserved during chronic infections of nonencapsulated *H. influenzae*. © 1997 Elsevier Science B.V.

Keywords: PCR; DNA sequencing; Protein D; Microbial pathogenesis

1. Introduction

Nonencapsulated *Haemophilus influenzae* causes acute respiratory tract infections and persistent infections in the lower respiratory tract of patients with chronic bronchitis and cystic fibrosis (van Alphen, 1992; Murphy and Sethi, 1992; van Alphen et al., 1995). The major outer membrane proteins (MOMPs) P2 and P5 of nonencapsulated *H. influenzae* are important antigens. Since the composition of MOMPs P2 and P5 is very heterogeneous and shows antigenic drift during persistent infections in patients with chronic bronchitis (Duim et al., 1994), the application of these MOMPs as vaccine components seems to be restricted.

Protein D is a 42 kDa lipoprotein of *H. influenzae* which implies that protein D may have a major periplasmic domain (Munson and Sasaki, 1993).

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Abbreviations: aa, amino acid(s); bp, base pair(s); hpd, gene encoding protein D; IgD, immunoglobulin D; kDa, kilodalton(s); MOMP, major outer membrane protein; nt, nucleotide(s); PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; SDS, sodium dodecyl sulfate.

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templates of the P5 gene were amplified by PCR. A cell lysate of
the strains were analyzed in the same way. From sputum
samples of the four patients with chronic bronchitis
from whom the strains A850053, A850047, A850079 and
A850048, respectively, were recovered (Groeneveld et al.,
1988; Duim et al., 1994) variants were isolated that
were genotypically identical but showed differences
in mobility of their MOMP P2 and/or P5 proteins. The
genotyping was performed by analysis of DNA finger-
prints after digestion with two restriction enzymes
(Groeneveld et al., 1988) and by random amplified
polymorphic DNA (RAPD) typing (van Belkum et al.,
1994). Time intervals between isolation of strains and
their variants were 9, 11, 8 and 3 months, respectively.
Although genetic variation was observed in their MOMP
P2 and P5 genes the H. influenzae MOMP variants
showed no differences in their hpd coding sequences
(Duim et al., 1994, 1996).

2.2. The nature of the hpd sequence variation

For occurrence of mutations an equal probability of
synonymous (silent) or nonsynonymous substitutions
(aa altering) is expected. The number of changes is
dependent on the number of synonymous and nonsynon-
ymous sites present in a given nt sequence (Sharp, 1991).
The numbers of synonymous and nonsynonymous nt
substitutions was calculated, using the method of Nei
and Gojobori (1986). The differences in the hpd genes
of four distinct H. influenzae strains were mostly
synonymous substitutions instead of nonsynonymous
substitutions (Fig. 1). Such a result is normally found
in bacterial genes that suffer from some functional or
structural constraints (Sharp, 1991).

2.3. Secondary protein structure prediction

Using the method of Garnier et al. (1987) for second-
ary protein structure prediction based on the deduced
sequence alignment of the hpd sequences of the four inde-
pendent H. influenzae strains revealed only minor nt dif-
fences (Fig. 1). Their hpd sequences were 96.3% identical,
two strains possessed identical hpd sequences, the other
two strains differed by 14 and 32 nt substitutions,
resulting in 2 and 5 aa changes, respectively. As the
C-terminal part of protein D is suggested to contain the
surface-exposed IgD-binding region, this region may
experience a selective pressure caused by the immune
system of the host and could be more variable than the
N-terminal part. However, the aa differences were ran-
domly distributed along the gene. The hpd gene
sequences were very similar to those reported by Song
et al. (1995), indicating that the nt sequences of the hpd
genes and consequently the deduced aa sequences, of
different nonencapsulated H. influenzae, including H.
influenzae type b are highly homologous independent
of their source.

Next, the hpd gene of four H. influenzae strains
A860509, A860501, A860503 and A850080 of the four
Table 1

| Name | Position in the hpd gene | Sequence (5’−3’)]
|------|-------------------------|---------------------
| hpd1 | 58−78                   | TGT AAA ACG ACG GCC GCT ATG AGC CAT TCA TCA AATATG |
| hpd2 | 385−403                 | TGT AAG ACG ACG GCC GCT CAT GCC AAA CAA GCG CAA G |
| hpd1r| 1086−1068               | CAG GAA ACA GCT ATG ACC TCC TTT TTA GAA TCC CAC G |
| hpd2r| 741−721                 | CAG GAA ACA GCT ATG ACC TCT TACC AAT CGT TAT AAG C |

Numbering derived from the published sequence of the hpd gene of H. influenzae type b (Janson et al., 1995).

The 5’ and 3’ ends of the primer sequences (TGT AAA ACG ACG GCC GCT ATG AGC CAT TCA TCA AATATG; TGT AAG ACG ACG GCC GCT CAT GCC AAA CAA GCG CAA G) were added at the
5’ and 3’ ends of the PCR products to allow direct sequencing of the PCR products using fluorescently labeled M13 primers (Duim et al., 1993). Oligonucleotide primers used for amplification and sequencing of the hpd

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Fig. 1. Comparison between hpd gene sequence and deduced aa sequence of four distinct nonencapsulated *H. influenzae* strains. PCR fragments obtained in two independent PCR reactions were sequenced in both directions using the Taq Dye Primers sequencing system with fluorescent dye-labeled −21m13 and M13 reverse sequence primers (Applied Biosystems). The primer sequences are underlined. DNA analysis was performed with an automated fluorescent DNA sequencer, Model 370A, Applied Biosystems. The derived sequences were analyzed using computer programs included in the program package Pcgene (Intelligentics, Inc., 1991). The nt sequences will appear in the EMBL/GenBank/DDBJ Nucleotide Sequence Databases under the accession Nos. X90493, X90489, X90491, X90485.
References


